Infrared spectroscopic characteristics of cell cycle and cell death probed by synchrotron-based FTIR spectromicroscopy

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INTRODUCTION

Conventional Fourier Transform Infrared (FTIR) spectroscopy and spectromicroscopy has been widely used as a diagnostic tool for characterizing the composition and structure of cellular components within intact tissues.[1-5] However, the spatial resolution of traditional FTIR spectromicroscopy is limited to ~75 μm with sufficient signal-to-noise at reasonably short data collection times.[6, 7] Synchrotron radiation-based FTIR spectromicroscopy (SR-FTIR), on the contrary, provides several hundred times higher brightness at a nearly diffraction-limited spatial resolution of 10 μm or better (depending on wavelength), and is therefore a sensitive analytical technique capable of providing molecular information at a significantly finer spatial resolution on biological specimens.[6-11] With this 10 μm or smaller spot size, SR-FTIR is ideally suited for the non-destructive, *in situ* study of processes that are taking place in individual cells. In this study we use SR-FTIR spectromicroscopy to investigate the spectral changes that occur in individual living human lung cells as a function of cell cycle and cell death.

EXPERIMENTAL DETAILS

The normal human fetal lung fibroblast IMR-90 P4 (passage 4) cell line was used for this study. They were cultured and grown to confluence on a plastic petri dish, as shown in the photograph in Figure 1(a). Cells were rinsed twice in PBS, scraped in 5mls media, mixed, and pipetted onto dried cleaned gold-coated glass slide pieces in 100 μl aliquots ($2x10^4$ cells) which were spread out with the pipette tip to insure single cells could be visualized. A photomicrograph of cells ready for infrared analysis is shown in Figure 1(b).

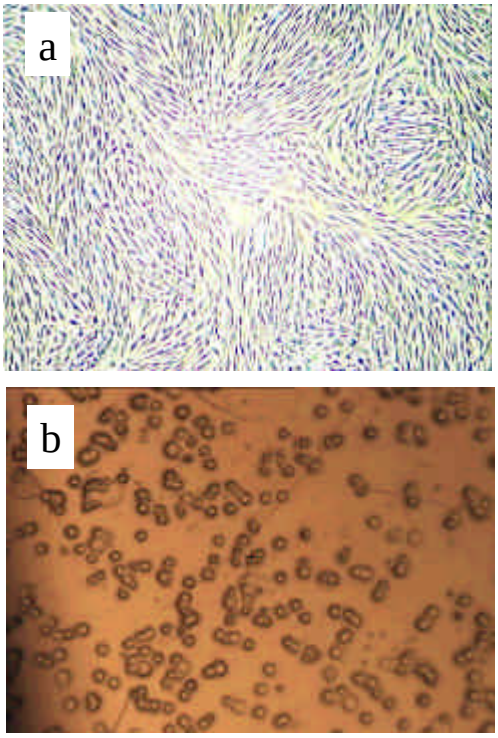

Figure 1. (a) IMR-90 cells grown to confluence, and (b) cells placed on gold-coated slides ready for SR-FTIR analysis.

Fluorescence-activated cell sorting (FACS)[14-16] was used to determine the distribution of cells in each phase of the cell cycle. The results of the FACS analysis showed 82.2% G1 phase, 9.3% S phase, and 8.5% G2/M phase. No cells of abnormal DNA content were observed.

Spectromicroscopic measurements were made at beamline 1.4.3 at the Advanced Light Source.[8, 17-19] The cells on gold coated glass were inserted into a small chilled chamber with a thin IR-transparent ZnSe window to maintain a more constant humidity, and prolong cell viability. This chamber was placed on the motorized microscope stage. Most of the liquid medium remaining on the slide surface was removed by carefully holding the slide at an angle against a piece of dry sterile gauze.

Data for this study was acquired in the double-pass transmission mode (the IR beam passes through a cell and is reflected back from the gold surface through the cell again). The sample stage was moved to align the center of the cell of interest with the focussed SR-IR spot to within a few microns. Typically spectra were obtained from an individual cell using 4 cm^{-1} resolution and 64 co-added interferograms. On the order of 100 individual cells were measured, spread through the various morphologies.

RESULTS

The cell division cycle consists of four major stages denoted Gap 1 (G1), Synthesis (S), Gap 2 (G2), and Mitosis (M) phases[20]. During S phase the DNA is replicated to form two complete copies of the cell's genes in preparation for division during the M phase. Visual inspection of cell size and morphology in the IR microscope at 320× magnification allowed tentative identivication of cells in G1, S and G2/M phases. We found that these cells showed clearly different infrared spectra. Figure 2 shows the unnormalized spectra in the 1800 to 900 cm^{-1} region for typical individual cells in each of these three phases. During S phase the DNA is undergoing replication and we observe that the absorptions in the DNA/RNA spectral region increase relative to the G1 phase spectra by approximately a factor of two. We also note that the centroid positions of Amide I and II peaks shift down in energy.

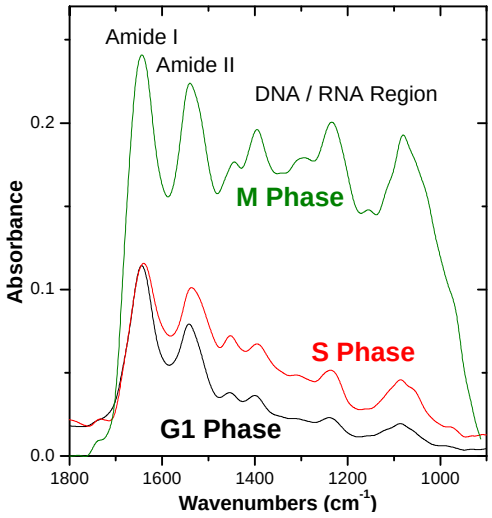

Figure 2. IR spectra of individual cells in different stages of the cell cycle. Spectra were not normalized, but a linear baseline was subtracted over the range of 2000 to 650 cm^{-1}.

When a G2/M phase cell is measured we observe a large increase in the overall absorbance (uppermost spectrum in Figure 2). This may be a result of more material in the cell, or because the thickness may be different in the M phase. In the second case there could be a greater path-length for the IR beam to traverse. Other more complicated mechanisms may also be active including the condensation of chromatin during these phases of the cell cycle. Absorptions in the DNA/RNA region are significantly increased relative to the protein peaks. It is also noteworthy that the peak around 1395 cm^{-1} is noticeably larger in the G2/M phase than in the other phases.

Occasionally some cells exhibited signs of dying or death even though visually the cell's morphology did not appear different. The spectrum of one such cell is shown in Figure 3 along with the spectrum of a normal living G1 phase cell. The "dying" cell shows two characteristic spectral signatures indicative of death.[12, 21] First, the centroids of the protein Amide I and II peaks shift from 1644 to 1633 and from 1542 to 1531 cm^{-1}, respectively, indicating a change in the overall protein conformational states within the cell. Second, we observe the appearance of a peak around 1743 cm^{-1}. These observations can now be used as signatures of cell death in future studies.

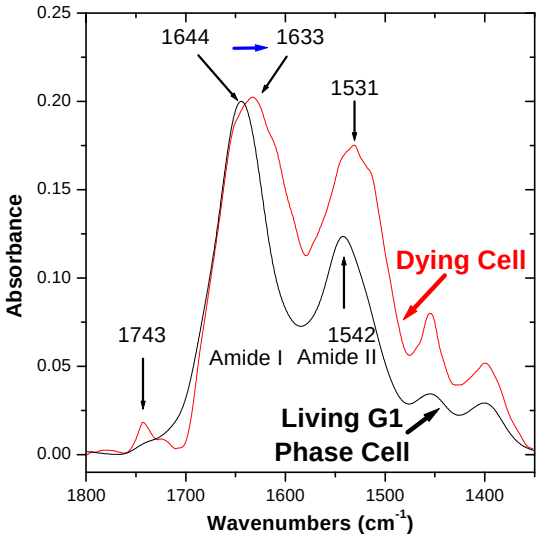

Figure 3. IR spectra of individual living and dying cells. Spectra were normalized to the Amide I peak, and a linear baseline was subtracted from 1800 to 1350 cm^{-1}.

We also measured a number of cells that were visually seen to have non-intact cell membranes. Spectra from these cells were similar to those reported by Jamin et al.[12,21] in that a peak at ~1728 cm^{-1} became prominent with a shoulder at ~1743 cm^{-1}. The Amide peaks were also observed to shift down to lower energies with the exact amount of the shifts varying from cell to cell.

A more complete analysis and discussion of these spectral observations is available in our published paper: Holman, *et al.*, Biopolymers, **57**[6], 329-335 (2000).

CONCLUSIONS

We have presented infrared spectra obtained from individual living human lung fibroblast cells as a function of cell cycle phase as well as for a cell that was dying or dead. We found very significant differences in the spectra which need to be understood and taken into account when making comparisons of IR spectra from normal and moribund cells. The power of using a synchrotron based IR source was demonstrated where data from one individual cell without any fixing, staining, or labeling provides excellent signal to noise. More importantly, we have shown that these single cell SR-FTIR spectra are of sufficient quality and reproducibility to allow detailed interpretation in terms of specific molecular events.

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